### Figure 1 Clone C35

#### A. DNA Coding Sequence

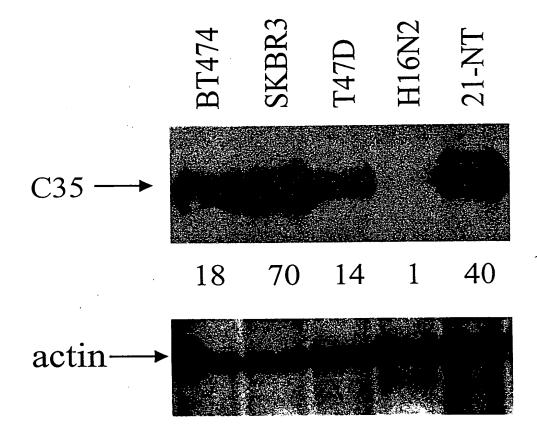
gcc gcg ATG AGC GGG GAG CCG GGG CAG ACG TCC GTA
GCG CCC CCT CCC GAG GAG GTC GAG CCG GGC AGT
GGG GTC CGC ATC GTG GTG GAG TAC TGT GAA CCC
TGC GGC TTC GAG GCG ACC TAC CTG GAG CTG GCC
AGT GCT GTG AAG GAG CAG TAT CCG GGC ATC GAG
ATC GAG TCG CGC CTC GGG GGC ACA GGT GCC TTT
GAG ATA GAG ATA AAT GGA CAG CTG GTG TTC TCC
AAG CTG GAG AAT GGG GGC TTT CCC TAT GAG AAA
GAT CTC ATT GAG GCC ATC CGA AGA GCC AGT AAT
GGA GAA ACC CTA GAA AAG ATC ACC AAC AGC CGT
CCT CCC TGC GTC ATC CTG TGA

#### B. Protein Sequence

MSGEPGQTSVAPPPEEVEPGSGVRIVVEYCEPCGFEATYLEL ASAVKEQYPGIEIESRLGGTGAFEIEINGQLVFSKLENGGFPY EKDLIEAIRRASNGETLEKITNSRPPCVIL\*

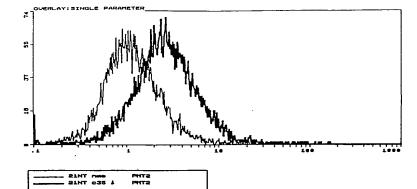
Z Figure 2. C35 is Expressed at High Levels in Breast Tumors but Not Normal Tissues 45 25 10 Actin **C35**  $BB\Gamma$ ГЛИС **PLACENTA** SMALL INTESTINE LIVER **KIDNE**A SPLEEN SUMYHT TJUQA СОГОИ SKELETAL MUSCLE HEART BRAIN 96 hr  $\overline{\mathbf{m}}$ 0.5 1 23 11 12 15 I° TUMOR 21PT I° TUMOR 21NT MET. TUMOR 21MT2 **MET. TUMOR 21MT1** NOKWAL BREAST LINE 3 WEEK THYMUS 0.8 kb

Figure 3

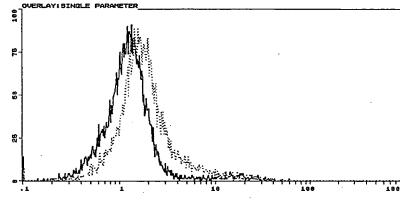


### Figure 4



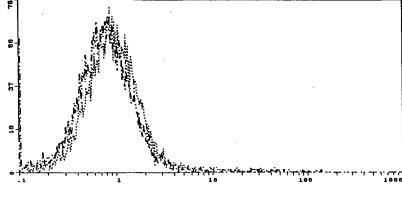


**B.** 



SKBR3 NS PMT2

C.



...... MDA c35 im PHT2

Figure 5A

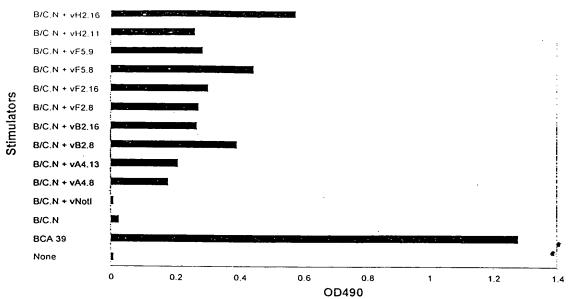


Figure 5B

	Percent Specific Lysis				
		: Target			
Target BCA 34	<u>10:1</u> 68.4	2:1 54.8			
BCA 39	36.6	23.4			
B/C.N	0.2	0.3			
B/C.N + vF5.8	47.5	34.6			
B/C.N + vH2.16	67.8	56.2			
B/C.N + vaccinia vector	0	0.2			

#### Figure 6

Α.	<u>L3</u>													
I	Amino Acid Position	45	46	47	48	49	50	51	52	53	54	55	56	
	Sequence	A	F	L	G	Y	K	Α	G	М	T	н	I,	
	Nucleotide	GCC	ттт	CTG	GGT	TAC	AAG	GCT	GGC	ATG	a <b>C</b> C	CAC	ATC	
В.	H2.16													
i	Amino Acid Position	45	46	47	48	49	50	51	52	53	54	55	56	
	Sequence	A	F	L	G	Y	к	A	G	M	I	Н	I	
	Nucleotide										- <b>T</b> -			

# Figure 7A Percent Specific Lysis

#### Effector: Target

Target	<u>10:1</u>	2:1
BCA 34	62.4	32.1
BCA 39	49.7	23.6
B/C.N	3.3	0.2
B/C.N + L3 peptide 48-56(I54)	46.0	16.1
B/C.N + L3 peptide 48-56(T54)	2.0	0
B/C.N + L3 peptide 45-54(I54)	0	0

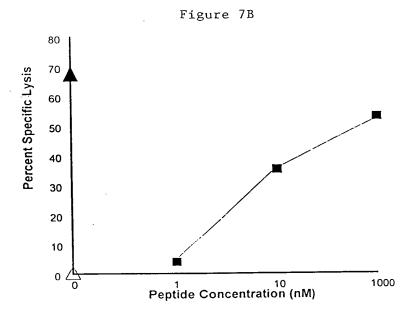
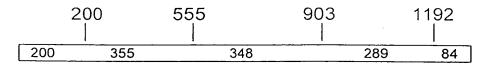


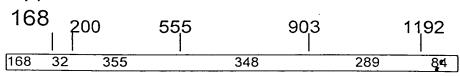
Figure 8A

### Published L3 (1276 bp)



168-171 = GACC

H2.16 (1276 bp)



168-171 = GATC

Figure 8B

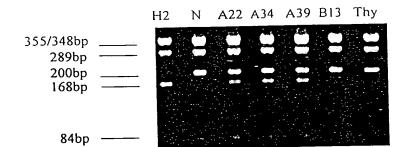


Figure 8C

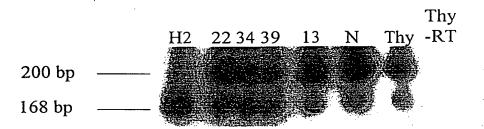


Figure 9A

# Percent Specific Lysis Immunogen v7.5/tk

	vH2	2.16	V1.3/LK			
Target	40:1	10:1 12.9	<u>40:1</u> 5.7	10:1 4.0	,	
BCA 34 BCA 39	33.6 22.1	9.0	5.3	3.1		
B/C.N + L3 48-56 (I54)	48.2	20.2	3.9	1.5		
B/C.N + L3.48-56 (T54)	6.4	1.4	1.8 6.1	2.9		
B/C.N YAC	7.1 1.2	5.7 2.5	0.1	1.8		
IAC						

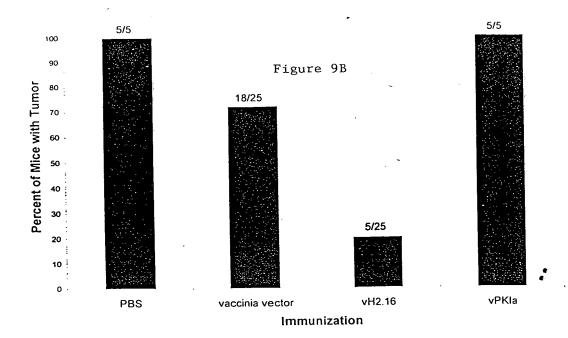
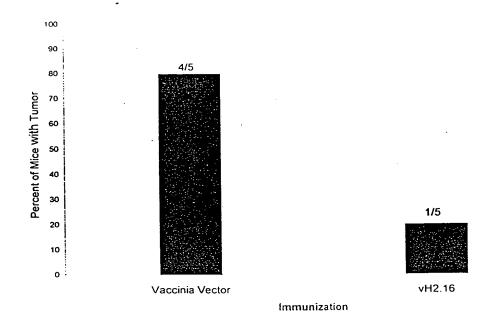


Figure 9C



Α.

gcccgagcggagccggcg ATG AGC GGG GAG CCG GGG CAG ACG TCC S Μ S G Ε Ρ G T 0 GTA GCG CCC CCT CCC GAG GAG GTC GAG CCG GGC AGT GGG GTC CGC V Α Ρ Ē Ρ Ρ  $\mathbf{E}$ V E Р G S G R ATC GTG GTG GAG TAC TGT GAA CCC TGC GGC TTC GAG GCG ACC TAC Y С C Ε Ε Ρ G F Ε Α Y CTG GAG CTG GCC AGT GCT GTG AAG GAG CAG TAT CCG GGC ATC GAG Α S Α V K Ε Q Y Ρ G Ε  $\mathbf{L}$ Ι ATC GAG TCG CGC CTC GGG GGC ACA GGT GCC TTT GAG ATA GAG ATA G Т Ι Ε S L G G Α F Ε Ι Ε Ι R AAT GGA CAG CTG GTG TTC TCC AAG CTG GAG AAT GGG GGC TTT CCC F S K L N G Р Q Ε G TAT GAG AAA GAT CTC ATT GAG GCC ATC CGA AGA GCC AGT AAT GGA Ε K D L Ι Ε Α Ι R R Α Ν G S GAA ACC CTA GAA AAG ATC ACC AAC AGC CGT CCT CCC TGC GTC ATC Τ  $_{\rm L}$ Ε K Ι Τ Ν S R Ρ Ρ  $\Box$  $\overline{V}$ I CTG TGA ctgcacaggactctgggttcctgctctgttctggggtccaaaccttggtct L

#### В.

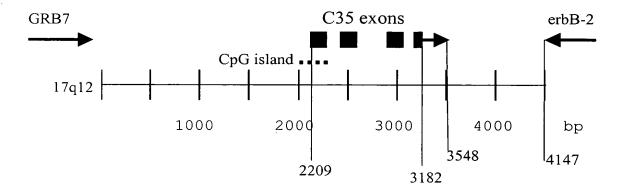
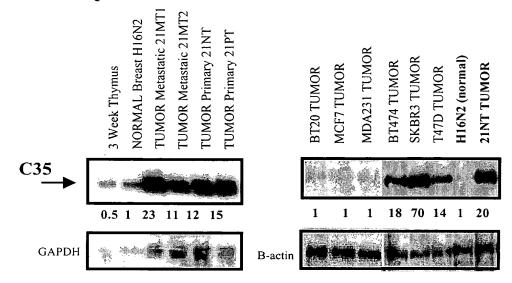


Figure 10

#### A. Breast epithelial cell lines



#### B. Primary breast tissue/tumors

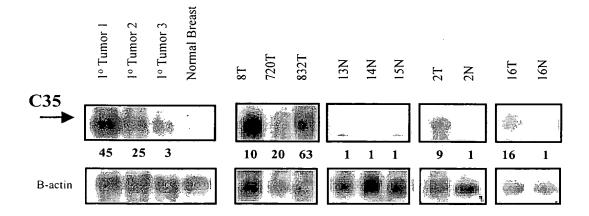


Figure 11

#### **Primary bladder tumors**

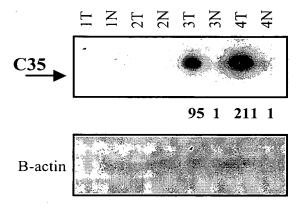


Figure 12

#### A. BREAST MDA-MB231(C35-) 21NT (C35+) **SERA** Pre-immune C35 imm. Pre-immune C35 imm. 21NT (C35+) H16N2 (C35-) **2C3 MAb** Events 10<sup>2</sup> FL4 10<sup>2</sup> FL4 103 10<sup>3</sup> **B. BLADDER** T24 SV-HUC ΜI (bladder tumor) PPT11A3 8. MI (Bladder normal) (bladder tumor) 9 M2 Counts 20 M2 M2

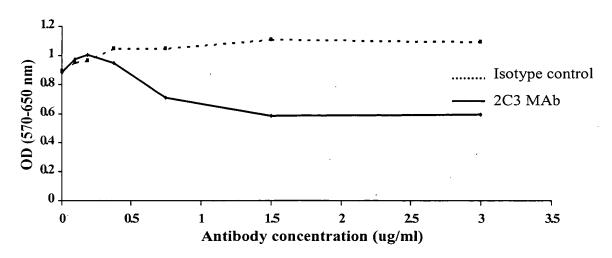
Figure 13

10<sup>2</sup> iso FITC

10<sup>2</sup> iso FITC

10<sup>2</sup> iso FITC

#### 21NT Breast Tumor



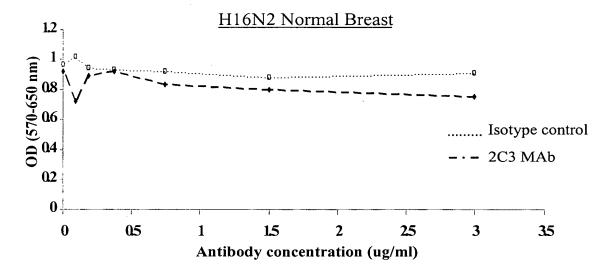
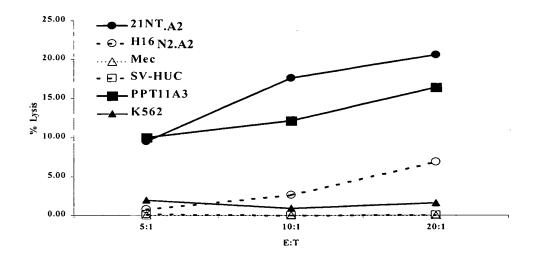


Figure 14

#### A. Lytic activity of C35-specific T cell line 4



#### B. Lytic activity of C35-specific T cell clone 10G3

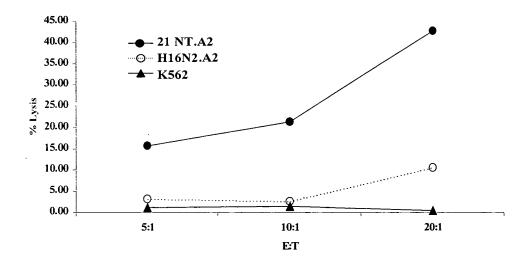
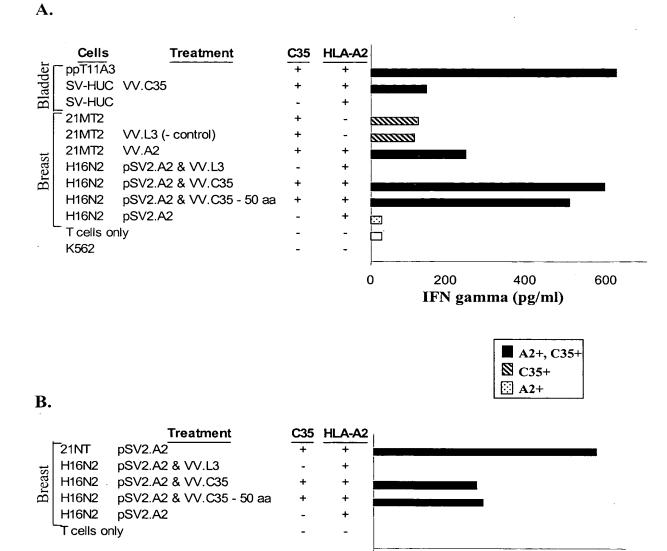


Figure 15



0

100

TNF alpha (pg/ml)

200

Figure 16

## Tolerance to Alloantigens Induced in presence of Antigens and Anti-CD40 Ligand Antibody

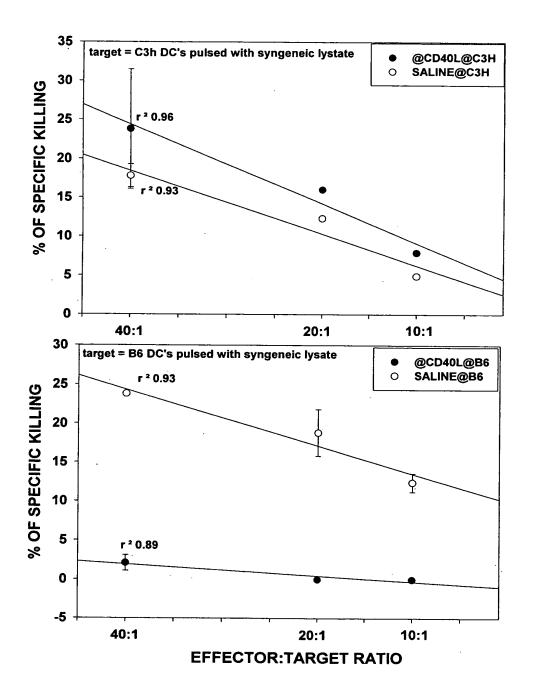


Figure 17